New method for NO detection using NMR Spin Trapping approach and fluorine-containing nitronyl nitroxides

Andrey Bobko[1], Sergey A. Popov[2], Vladimir A. Reznikov[3], Elena G. Bagryanskaya[2], Thomas L. Clanton[5], and Valery V. Khramtsov[4, 5]

[1]Novosibirsk State University, Novosibirsk 630090, Russia

[2]International Tomography Centre, Novosibirsk 630090, Russia

[3]Novosibirsk Institute of Organic Chemistry,
Novosibirsk 630090, Russia

[4]Institute of Chemical Kinetics and Combustion, Novosibirsk, 630090, Russia

[5] Dorothy M.Davis Heart Lung Research Institute, Division of Pulmonary and Critical Care Medicine, The Ohio State University, Columbus, OH

Recently we proposed ^{19}F-NMR Spin Trapping approach for the detection of free radicals [1]. In the present data we expand this NMR method for detection of nitric oxide (NO) using nitronyl nitroxyl radicals (NNR) or their related hydroxylamines. For this purpose series of new fluorine-containing NNR (2-substituted (p-trifluorophenyl (NNR1), m-difluorophenyl (NNR2), or trifluorometyl (NNR3)) – 4,4,5,5-tetrametyl-2-imidazoline-3-oxide-1-oxyl) have been synthesized. It has been shown that the NNR and corresponding INR have different EPR and ^{19}F-NMR spectra. Thus hyperfine structure derived from interaction with two nitrogen and 3 equivalent fluorine atoms has been observed both for NNR3 and corresponding INR3 while their magnitudes significantly differ ($a_{N1}=a_{N3}=7.12$ G, $a_F=3.44$ G for NNR3 and $a_{N1}=8.48$ G, $a_{N3}=4.39$ G, $a_F=1.76$ G for INR3). Corresponding ^{19}F-NMR spectra of NNR and INR also significantly differ showing single resonances at 73 ppm for NNR3 and at 78.5 ppm for INR3, respectively. The reaction of the NNR1-NNR3 with NO has been tested both by EPR and NMR in several NO generating systems (using NO donors such as S-Nitroso-N-acetylpenicillamine and 3,4-dihydrodiazete 1,2-dioxides, or adding NO-saturated solution) both in the presence and absence of reducing agents. It has been shown that EPR and ^{19}F-NMR spectra of observed reaction products of the NNR1-NNR3 and NO coincide with the spectra of corresponding INR. In summary, the data observed demonstrates a new approach for NO detection using NMR spin trapping and fluorine-containing NNR. Taking into account the well-developed NMR imaging technique this method has potential to be applied for imaging of NO in vivo.

This work was partly supported by RFFI grant NU-02-04-48374 (Russia) and USPHS GM58772.

Khramtsov, V.V. et al. 2001, *Free Rad.Biol.Med*. 30 (10): 1099-1107.